

Effect of water on micronutrient content and yield in rice (*Oryza sativa* L.)

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ABSTRACT

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Rice (*Oryza sativa* L.) is an important crop in many countries. According to the IRRI, more than 2 billion people globally depend on rice as a staple food (Dawe, et al. 2003). Studies on micronutrient content in rice and the effect of water in availability of the nutrients may aid in decreasing global nutrient deficiencies. Rice is grown under different water regimes such as AWD and intermittent flooding, sprinkler and furrow irrigation. A greenhouse pot experiment of rice utilizing a split plot design under different water regimes was conducted to assess the affect of water on (1) DTPA extractable soil micronutrients Fe, Mn Cu and Zn; (2) stem micronutrient concentration and uptake of rice (*Oryza sativa* L.) alone and with ground legume incorporated; and (3) yield of rice. The water regimes included: (1) rice pots watered to field capacity, or well-drained (drain); (2) pots submerged for 3 weeks, dried down for 1 week, then re-submerged for three weeks, or alternate wet and dry (AWD); and (3) continuous flooded (flood) conditions. A preliminary study which examined vegetative yield and micronutrient uptake of rice, faba beans, and sesbania using two different soils (Zaca clay and a loam) under flooded and drained conditions was conducted. Ground sesbania from the exploratory experiment was used for the study to explore the effects of organic matter (OM) on yield and micronutrient content.

All DTPA extractable soil micronutrients except Zn were highest in the flood and AWD water treatments and in the lowest pH value. The DTPA extractable Zn values in the drain water treatment were twice as high as the AWD and flood water treatments.

Stem Zn concentration was highest in drained, whereas Fe, Mn and Cu stem concentration were highest in AWD and flood treatments. Addition of sesbania incorporated into the soil only affected Mn soil micronutrient concentration, where Mn soil content was observed to be higher in the flood treatment with sesbania incorporated into the soil.

Grain weight and grain to stem ratio were significantly increased by AWD and flood water treatments ($p < 0.05$). Organic matter (sesbania) incorporated into the soil did not affect Fe, Mn, Cu or Zn stem to grain ratio.

A comparison of stem concentration to grain yield highlighted the effects of Mn and Zn content on yield. Manganese stem content was highest in AWD and flood treatments where grain yield and grain to stem ratio were highest, while Zn content was lowest in AWD and flood treatments. Zinc stem content was highest in the drain treatment.

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CHAPTER ONE

INTRODUCTION

Understanding wetland soils and plants is becoming increasingly important in a world where water either may become scarce or excessive. As climate changes affect natural and cultivated plants, the need for scientific data and information on plant physio-chemical behavior becomes increasingly more important. Crop-soil-water systems depend on nutrient balance. In the wetlands, for example, plants act in unison with soil, water and microorganisms to adapt to the saturated conditions.

Rice is the most widely grown wetland crop in the world. Agencies such as the International Rice Research Institute (IRRI.org) help farmers throughout the world learn more about rice and other grain crop nutritional needs. Research conducted by IRRI and other agencies also focus on water saving strategies. The IRRI and US agencies such as USDA monitor rice production throughout the world.

The IRRI (2009) provides FAO production, area, and yield data. World rice production in 2008 was 661, 811,000 tons (IRRI, 2009). There are approximately 114 countries which grow rice, and more than 50 countries have an annual production of over 100,000 tons (McClean et al. 2002). Farmers in Asian countries produce about 90% of the total, with two countries, China and India, growing more than half the total crop (IRRI, 2009). In California, total rice production was 2,151,500 tons in 2008. The United States produced about 10,186,650 tons in 2008 (NASS, USDA, 2009).

Rice can be grown in rotation with other grains such as wheat, maize or with legumes for nitrogen fixation value. Straw residue incorporation is a desired cropping technique to improve soil, nutrient availability and other qualities. In rice, as well as with other cropping systems, legumes add nitrogen to the system and can enhance

micronutrient absorption. The legume, *Sesbania spp.* is considered a weed in the U.S., and not used as a rotation crop for rice. In Asian countries, sesbania is grown as green manure crop to enhance nitrogen fixation. It is a semi-aquatic plant and has a high tolerance to flooding. Regular incorporation of sesbania green manure before rice transplanting has been shown to improve rice yield and increase micronutrients, particularly Fe and Mn (Nayyar and Chhibba, 2000). Proper management of rotational crops and co-crops is important to avoid nutrient interactions and toxic organic acid affects (Yoshida, 1981).

Irrigation regimes in wetland crops are diverse and can be customized by farmers to facilitate maximum micronutrient supply. Irrigation regimes include continuous flood, alternate wet and dry, partial submergence, and sprinkler irrigation. The technique known as alternate wetting and drying (AWD), is used in paddy fields to reduce water consumption (Belder et al., 2004), reduce the incidence of malaria due to fewer mosquitoes, reduce methane production, and improve grain quality based on sucrose to starch conversion.

An exploratory experiment was conducted with rice (*Oryza sativa* L.) and an organic mix of sesbania (*Sesbania macrocarpa* Muhl.) and faba beans (*Vicia faba* L.) to investigate micronutrient concentrations and plant absorption under two different water regimes and using two different soils (Zaca clay and loam) The ground sesbania from the exploratory experiment was used as the organic matter incorporated into the soil for this experiment.

The objectives of this study were to investigate the effect of three different water regimes on soil and stem micronutrient concentrations and to investigate the effect of

organic matter (sesbania) incorporated into the soil on soil and stem micronutrients and rice yield.

CHAPTER TWO

REVIEW OF LITERATURE

Micronutrients in the soil

Nutrient movement through the soil

The three ways nutrients move through soil are interception, mass movement and diffusion (Barber, 1984). Root interception, or contact absorption (Troeh and Thompson, 2005) is the ability of the root to grow into the soil. Roots uptake the nutrients as they grow. Mass flow is the movement of nutrients in the soil with water flow. Mass movement may be calculated by multiplying water use per plant by the concentration in the soil solution (Barber, 1984). Movement by diffusion occurs when a concentration gradient exists, causing a greater movement from the zone of higher concentration to the zone of lower concentration, creating a net movement toward the zone of lower concentration (Barber, 1984).

Mineral forms

Iron (Fe) is the fourth most abundant element, comprising about 5% of the earth's crust (Havlin et al., 2005). The range of iron in soil is estimated to be between 10,000 to 100,000 ppm (Brady and Weil, 2004). Iron forms include oxides, sulfides and silicates. Olivine $[(Mg,Fe)_2SiO_4]$, siderite $(FeCO_3)$, hematite (Fe_2O_3) , goethite $(FeOOH)$, and magnetite (Fe_3O_4) make up the common primary and secondary minerals (Brady and Weil, 2004). Iron soluble inorganic forms include Fe^{3+} , $Fe(OH)_2$, $Fe(OH)_2^+$, Fe^{2+} , $Fe(OH)_3^-$, and $Fe(OH)_4^{2-}$ (Kabata-Pendias and Pendias, 1984).

Next to Fe, Manganese (Mn) is the most abundant and important micronutrient (Havlin et al., 2005). The average Mn soil content is 510 ppm to 1,000 ppm for various soils around the world with a maximum Mn content of 4000 ppm (Adriano, 1986). The world mean is 450 ppm. Mn content (Adriano, 1986). When Mn is released through weathering of primary rocks, it becomes oxidized to form secondary minerals, including

pyrolusite (MnO_2), hausmannite (Mn_3O_4), and manganite (MnOOH). Pyrolusite and manganite are the most abundant forms of Mn (Havlin et al., 2005).

Manganese mainly exists in three valence states, Mn^{4+} , Mn^{3+} , and Mn^{2+} . The most common form in solution is Mn^{2+} . Its concentration decreases a hundredfold for each incremental unit increase in pH. Organically complexed Mn^{2+} comprises about 90% of solution Mn^{2+} . It is mainly controlled by MnO_2 and ranges from approximately 0.0 to 10 ppm (Havlin et al., 2005). Manganese is reduced from Mn^{4+} or Mn^{3+} to Mn^{2+} by bacteria within weeks in most soils (Ponnamperuma, 1972). Manganese in soil solution (Mn^{2+}) increases under acidic conditions and can leach from coarse-textured soils. Above pH 8.5, solubility of Mn^{2+} is critically low (Kyuma, 2004).

Zinc (Zn) is another important micronutrient found in soil, although it is found in smaller amounts than Fe and Mn. Zinc content in soil is about 80 ppm and Zn in soil ranges from approximately 10 to 300 ppm and averages 50 ppm (Havlin et al., 2005). Zinc is found in igneous rocks at levels of approximately 70 ppm, sedimentary rocks (shale) at approximately 95 ppm, limestone at approximately 20 ppm, and sandstone at approximately 16 ppm (Havlin et al., 2005). Franklinite (ZnFe_2O_4), smithsonite (ZnCO_3) and willemite (Zn_2SiO_4) are common minerals which contain Zn. (Havlin et al., 2005). Unbound Zn occurs in soil as Zn^{2+} .

Copper is a micronutrient found in small amounts in soil, but important to plants. Copper concentrations in soil range from approximately 1 to 40 ppm. The reported world Cu average is 30 ppm (Adriano, 1986). Copper is found in the primary minerals malchite ($(\text{Cu}_2)\text{OH}_2\text{CO}_2$) and cupric ferrite (CuFe_2O_4) (Havlin et al., 2005). Oxides, carbonates, silicates, sulfates, and chlorites are secondary compounds containing Cu. In soil solutions, copper ranges between 10^{-8} and 10^{-6} M (Havlin et al., 2005).

Copper (Cu) sources include organic Cu in animal wastes and inorganic sources in the form of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, CuSO_4 and $\text{Cu}(\text{OH})_2$, and Cu chelates such as Na_2CuEDTA (Havlin et al., 2005).

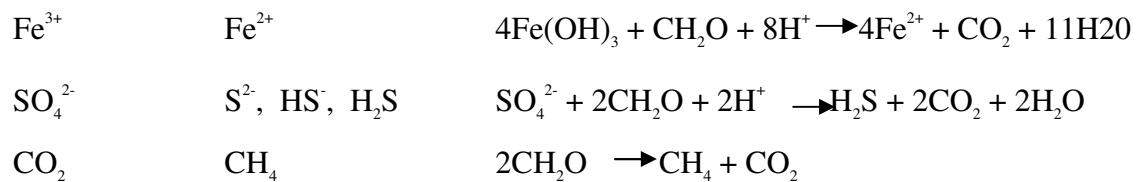
Copper is mostly found as Cu^{+2} at $\text{pH} < 7$ and CuO at $\text{pH} > 7$ (Havlin et al., 2005). Copper is chemically adsorbed to surfaces of clays, organic matter, and iron, aluminum or manganese oxides (Havlin et al., 2005).

Reduction processes in soil

Oxygen diffusion is about 10^3 - 10^4 times faster in air than in waterlogged soils and involves the process of respiration of soil microorganisms and plant roots (Marcher, 1995). Each process in reduction is a sequential process over time. The sequence of reduction takes place at specific redox potentials. In a saturated soil, oxygen is completely depleted in one day. The NO_3^- is then reduced. After reduction of NO_3^- , Mn and Fe reduction occurs. In the first 1 to 2 months of submergence, Fe (III) is reduced to Fe (II) (Ponnamperuma, 1972, Kirk, 2004).

Table 2.1. The oxidization and reduction processes of manganese, iron, sulfur, and carbon.

Oxidized Form	Reduced Form(s)	Reaction
O_2	CO_2	$\text{CO}_2 + \text{H}_2\text{O}$
NO_3^-	NH_4^+ , N_2O , N_2	$4\text{NO}_3^- + 5\text{CH}_2\text{O} + 4\text{H}^+ \rightarrow 2\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O}$
Mn^{4+}	Mn^{2+}	$2\text{MnO}_2 + \text{CH}_2\text{O} + 4\text{H}^+ \rightarrow 2\text{Mn} + \text{CO}_2 + 3\text{H}_2\text{O}$



Adapted from Wetland Plants: Biology and Ecology. Cronk and Fennessey, (2001) and Kirk, (2004)

The reduction of Mn (IV) is carried out by microbial species of genera *Bacillus*, *Clostridium*, *Micrococcus*, and *Pseudomonas*. Iron reduction utilizes microbial species of genera *Bacillus*, *Clostridium*, *Klebsiella*, *Pseudomonas*, and *Serratia* (Alexander, 1977).

Plant Mechanisms

Micronutrient absorption and transport systems

Plants utilize two transport systems to move ions through tissues. Short distance transport involves symplastic movement (diffusion from cell to cell but first through a plasma membrane then through plasmodesmata) and apoplastic movement (movement of materials through cell wall tissues). Short-distance transport at tissue level is lateral, usually along the radial axis of the plant, from the outer portion of the tissue to the inner portion (or conversely), and is diffusion driven.

Solute transport is facilitated by water potential. Water potential is described as osmotic potential plus hydrostatic potential, and by the formula $\psi = \psi_s + \psi_p$. **Water enters** and leaves cells along a gradient, from an area of higher water potential to one of lower water potential. Earnest Munich (1930) proposed that the pressure gradient is between source and sink and is established as a result of phloem loading at the source and unloading at the sink (Taiz and Zeiger, 2002).

In source tissues, sugars accumulate as a result of phloem loading, generating a low (negative) solute potential. Water flows into the sieve cells and causes turgor pressure to increase (Taiz and Zeiger, 2002). Phloem loading decreases sugar

concentration in the sieve elements, creating a higher solute potential in the sieve elements. Water potential in the phloem rises above that of the xylem, and subsequently leaves the phloem, causing a decrease in turgor pressure. It is by bulk flow that sieve element contents are moved along the translocation pathway (Taiz and Zeiger, 2002).

A transport strategy for plants involves either a strategy I or strategy II mechanisms. In strategy I plants (non-grasses), Fe^{3+} is reduced to Fe^{2+} . Iron deficient plants acidify the soil by a Fe^{3+} chelate-reductase, H^+ -ATPase, in the root epidermal cells (Curie and Briat, 2003). Strategy II plants (grasses) form mugineic acid ferric iron complexes (MA-Fe^{3+}) which allow chelation and transport under Fe deficient conditions (Sharma, 2006). Gries et al, (1998) observed phytosiderophore release in iron and copper deficient grass with the addition of Fe, Zn, Mn and Cu using solutions buffered with chelating nutrients. They observed reduced Fe, Zn, Mn, and Cu compared to the trace-metal sufficient control plants. Iron is transported from roots to the shoots via the xylem, along a transpiration stream. Ferric citrate is the mobile form of iron. Reduced iron (Fe^{2+}) is re-oxidized in the cytoplasm for long-distance transport (Sharma, 2006, Becker and Asch, 2005).

Phloem transport of iron takes place as ferrous iron in an organically bound form (Fe (II)) (Sharma, 2006). The main carrier of iron in the phloem is nicotianamine, an organic acid derived from methane.

Plants take up copper as Cu^+ , the monovalent state facilitating reduction by plasmalemma-bound cupric reductases (Sharma, 2006). This possibly involves the same plasma membrane reductase system which facilitates the reduction of Fe^{3+} (Sharma, 2006). Similar to iron, plants respond to copper deficiency by regulating root cell plasma membrane ferric reductase and inducing acidification (Havlin et al., 2005).

Manganese (II) uptake and transport occurs via the root cells through the plasmalemma by a specific transporter protein. This process establishes an electrical gradient where the cell wall is more positive (less negative) than the cell interior (Havlin et al., 2005). High concentrations of Ca^{+2} and Mg^{+2} taken up by apoplasmic cell walls, especially in high pH soils, can reduce Mn^{+2} uptake and transport in cell walls (Havlin et al., 2005). Manganese uptake increases through the process of proton pumping referred to as H^+ efflux, causing polarization of the plasmalemma, and decreases when the efflux of H^+ decreases into the cytosol (Sharma, 2006).

Zinc is taken up by plants in the form Zn^{2+} . It is concentration-dependent and is facilitated by carrier-mediated transport. Phytosiderophores are secreted by strategy II plants in response to Zn-deficiency. Zinc is phloem mobile and foliar-spraying plants can provide sufficient zinc for growth. Zinc is deficient in plants grown under submerged conditions (Yoshida, 1981).

Micronutrients role in plants and deficiency and toxicity symptoms

Iron is involved in photosynthesis II as ferredoxin in the electron transport system. Iron is contained in porphyrin molecules such as cytochromes, hemes, hematin, ferrichrome, and leghemoglobin, and in iron-sulfur proteins; and functions as isoenzymes of superoxide dismutase (SOD) (Marschner, 1995). Chloroplasts contain up to 90% iron in leaf cells.

Iron deficiencies of iron affect photosynthesis and decreased dry matter production. Deficiency occurs as interveinal chlorosis (loss of chlorophyll) in young leaves. Iron deficiencies occur mostly in upland soils under conditions including high pH

soils, high P to Fe ratio in the soil, and excessive concentrations of competing ions such as Mn, Cu, and Zn, and Al (Doberman and Fairhurst, 2002). Rice cultivars that are not entirely able to excrete organic acids to solubilize Fe also may be deficient in iron. Iron toxicities occur generally on lowland soils which remain permanently flooded. Toxicity symptoms include orange-yellow leaf tips, purple-brown appearing leaves, and stunted growth with severely limited tillering (Dobermann and Fairhurst, 2002).

The critical Fe soil toxicity concentration is greater than 600 ppm (Dobermann and Fairhurst, 2002). The Fe(II) concentration in the soil solution after 4 weeks of submergence concentrations can reach as high as 300 ppm (Yoshida, 1981), but then decreases exponentially. After 6 months submergence, Fe (II) concentration was maintained at approximately 50-100 ppm (Yoshida, 1981). Factors affecting Fe availability include soil pH and bicarbonate (HCO_3^-), redox potential, sodic conditions, organic matter, and submerged conditions.

Manganese is an early step in oxidation-reduction of submerged soils, is involved in the electron transport system and photosynthesis, and is a co-factor in several enzymes acting to catalyze oxidation-reduction, decarboxylation, and hydrolytic reactions (Marschner, 1995). In Mn toxicity, yellowish-brown spots appear and extend to the interveinal area, while brown spots appear on leaf blades and sheaths. In Mn deficiency the brown spots occur later and leaves become dark brown. Stunting occurs in both toxicity and deficiency, but with toxicity, tillering is reduced.

Copper contains proteins which facilitate enzyme activity such as oxidase, plastocyanin in photosynthesis, peroxidases, and ascorbate oxidases. Copper is also a component of superoxide dismutases (SOD) which detoxifies the superoxide radicals.

Copper is required for lignin synthesis and in the microsporogenesis stage of pollen formation.

Copper deficiency appears as chlorotic streaks on leaves, dark brown necrotic lesions on leaf tips, bluish green leaves, reduced tillering, and increased spikelet sterility with unfilled grains. Copper toxicity may induce Fe deficiency symptoms, observed as interveinal chlorosis on younger leaves (Marschner, 1995). Factors affecting Cu availability include texture. The highest likelihood of Cu deficiency is in highly leached, coarse-textured soils. Copper deficiencies are especially seen in lateritic, highly weathered soils such as ultisols and oxisols and sandy textured or calcareous soils (IRRI, 2002). Soil pH is also a factor; solution Cu decreases with increasing pH due to decreased mineral solubility and increased soil adsorption. Copper is also deficient in peat soils (Kyuma, 2004) due to chelating or complex formation with organic compounds. Critical levels for Cu are from 0.2-0.3 ppm, DTPA + CaCl_2 , pH 7.3 (IRRI, 2002). Reasons for Cu deficiency include strong adsorption of Cu on humic and fulvic acids, commonly seen in peat soils, small amounts of Cu in sandy soils with quartz as the parent material, large NPK fertilizer application rates, resulting in the dilution factor (Smith, 2007), and excessive Zn in the soil, competing with the Cu uptake (IRRI, 2002).

Copper toxicity affects rice roots more than shoots. Chen et al (2000) observed an increase in lipid peroxidation in rice roots when CuSO_4 was added to roots. Excessive Cu resulted in oxidative stress. Copper toxicity may induce Fe deficiency symptoms, observed as interveinal chlorosis on younger leave (Marschner, 1995). Copper toxicity is rare in rice and mostly occurs in soil exposed to wastewater containing high levels of Cu.

Zinc has a strong role in protein molecules involved in DNA replication.

Zinc is involved in enzyme activation such as alcohol dehydrogenase in flooded rice.

Zinc is also a component in superoxide dismutase. In CuZnSOD, the copper atom facilitates the catalytic metal component, and the Zn atom the structural. Other functions provided by Zn are membrane integrity by binding to phospholipids and sulfhydryl groups or the formation of tetrahedral complexes in polypeptide chains, protecting membrane lipids and proteins from oxidative damage (Marschner, 1995).

At high pH (> 7) Zn^{2+} becomes immobile and unavailable to plants due to incorporation of Zn in Ca and Mg carbonates (Kirk, 2004), or precipitation as insoluble amorphous Zn compound, ZnFe_2O_4 , or ZnSiO_4 (Havlin et al., 2005).

Symptoms of Zn deficiency include brown spots on upper leaves of stunted plants, reduced tillering, increased spikelet sterility loss of turgor, and white lines along the leaf midrib (Doberman and Fairhurst, 2002). High Zn content in stems can induce a Mn deficiency causing affected leaves to show reddish-brown necrotic spots (Marschner, 1995).

Critical soil zinc deficiency levels occur at 2.0 ppm Zn in 0.1 N HCl (IRRI 2002). Causes of Zn deficiency include high pH (>7.0 in submerged conditions), high bicarbonate concentration in reducing conditions in calcareous soils or irrigation water, and availability of cations (Fe^{2+} , Ca^{2+} , Mg^{2+} , Cu^{2+} , and PO_3^+) after submergence (IRRI, 2002).

Soils susceptible to Zn deficiency are leached, sandy, highly weather, acid, and soils with coarse textures. Submerged conditions decreases Zn availability due to increase in soil pH (Kyuma, 2004, Kirk, 2004). Planting at 12 weeks after soil submergence to allow HCO_3 and organic acids concentrations to decrease helped increase Zn uptake from 53 to 85 percent increase (Forno et al., 1974).

Zinc toxicity occurs in soils where Zn has been applied, most often in wastewater. Symptoms include stunting of growth and chlorosis. Plant tolerance to Zn toxicity is variety dependent (Dong et al., 2004)

Plant tolerance strategies to high micronutrient concentrations

There are three strategies plants utilize to tolerate high micronutrients concentrations. Accumulators (type I) are plants concentrating metals in above-ground plant parts through all soil levels, indicators (type II) are plants where absorption and transport of metals to the stem are in equilibrium with the external and internal levels, and excluders (type III) as plants where metal concentration in the stem are maintained at a critical point before the mechanism breaks down (Baker 1981). Mechanisms of tolerance in both types I and type III are largely internal and the difference is principally in the sites of detoxification. The excluders detoxify in the root whereas the accumulators detoxify metals in the stems (Baker, 1981)

Wetland rice species adapt well to high levels of manganese and iron. Manganese and iron exclusion power are responsible for this phenomenon. The iron excluding power of a rice plant is 87% (Yoshida, 1981). Thus, 87% of the iron reaching the root surface, along with the water absorbed by the plant, is not absorbed or not excluded. The formula is $(a-b/a) \times 100\%$, where a is the amount of iron in milligrams, in the same volume of solution as the water absorbed by the plant, and b is the amount of iron, in milligrams, actually absorbed by the plant (Yoshida, 1981).

Evidence exists in waterlogged plants of increased super oxidase dismutase (SOD) activity under anaerobic conditions as an important preventative mechanism in oxidative damage during recovery from anoxia stress. Hendry and Brocklebank (1985) examined a type II (accumulator) response to an accumulation of ferrous ion

concentration in *Epilobium hirsutum* L. by the formation of super oxide radical and the induction of SOD with the initial product H_2O_2 accumulating in the absence of catalase and low activity of root peroxidases. The result was a hydroxyl radical formation, inhibition of protein synthesis, increased lipid peroxidation and gross cellular damage (Hendry and Brocklebank, 1985). They concluded H_2O_2 must be degraded initially to prevent cell damage.

Root and stem mechanisms under submerged conditions

Wetland plants utilize special root mechanisms in order to survive in anaerobic conditions. Wetland species contain lacunae held together by porous aerenchyma tissue. In emergent wetland plants, gases enter the aerial parts of the plant through stomata in leaves and through lenticels in stems or woody plants. It travels toward the roots through the aerenchyma tissue by diffusion (Cronk and Fennessey, 2001). Carbon dioxide travels from the roots to the above ground portions of the plants and out through the stomata. Aerenchyma forms by separation of the cell wall (lysigeny) and by increasing size and separation of cells (schizogeny) (Cronk and Fennessey, 2001).

Aerenchyma decreases oxygen flow resistance in wetland plants. Aerenchyma allows ethylene and carbon dioxide to escape into the atmosphere as well as provide storage for gases. The more air space within the plant, the greater the storage capacity (Cronk and Fennessey, 2001). As reduced condition increase, the porosity of plant tissues increases. Lacunal space increases as sediment anaerobiosis increase in deep water *Oryza sativa* and other aquatic plants.

Roots develop organs in order to adapt to submerged conditions. The seminal roots emerge directly from the apical meristem of the embryo in their germinating seed, whereas nodal or adventitious roots subsequently emerge from successive nodes on the

stems (Tinker and Nye, 2000). Adventitious roots aid in water and nutrient absorption in flood-tolerant plants. Adventitious roots have aerenchyma, and possess a continuous network of pores throughout the entire root and stem system.

Management

Plant analysis to determine deficiencies and toxicities

Plant analysis is based on one of three systems-(1) sufficiency ranges: (2) The Diagnosis and Recommendation Integrated System (DRIS), and (3) Plant Analysis and Standardized Scores (PASS). Sufficiency ranges utilize a critical level, the concentration below which yields decrease and deficiency symptoms appear. It is commonly regarded as the nutrient concentration at 90 or 95% of maximum yield. Critical levels are analyzed for a specific plant anatomical part at a specific stage of maturity (Smith, 2007). For rice this analysis is performed on the Y-leaf at different stages. Nutrient contents are categorized as deficient, low, sufficient, high, and excessive concentrations. As plants go from the vegetative growth stage to the reproductive growth stage, the concentration of most nutrients decreases. This is known as the dilution factor (Smith, 2007) and guides the principles of sufficiency levels.

The Diagnosis and Recommendation Integrated System (DRIS) (Walworth and Sumner, 1987) is an index by which a database is created from analysis of thousands of samples of a crop. The highest yielding plants provide the standard used as the basis for comparison. A combination of different nutrient ratios is used to identify which nutrients are most likely to limit yield. The calculated results are DRIS indices. An index of 0 is considered optimum and indices ranging between -15 to +25 are considered normal and in balance. Large negative values are considered deficient while large positive values are considered in excess. Thus, the greater the magnitude of the nutrient index, the more

likely that element is sufficient or deficient. With DRIS, the stage of maturity, plant part, and cultivar are of less importance than are for the critical level or sufficiency range approaches of the sufficiency range approach.

Plant Analysis with Standardized Scores (PASS) was developed at the University of Wisconsin to combine the advantages of the Sufficiency Range and Diagnostic and Recommendation Integrated System (DRIS). The SR method provides independent nutrient indices of categorical data, and DRIS provides numerical dependence of nutrient indices. PASS combines an independent nutrient section and a dependent nutrient system. PASS has only been developed for alfalfa, corn and soybean.

Fertilizer amendments

Micronutrient availability varies among crops in different water regimes, soil types and soil pHs. Amendments properly applied will balance micronutrients and avoid toxicities or deficiencies.

Liming is considered beneficial in a macronutrient deficient system. In a micronutrient deficient system, even a small amount of over liming can create chlorosis symptoms. Liming materials include calcium oxide (CaO), Calcium Hydroxide (Ca(OH)₂), calcium and Calcium-Magnesium Carbonates (CaMg(CO₃)), and Calcium Silicates (CaSiO₃).

Acidifying may be needed for crops with a low optimum pH range grown on calcareous soils. The CaCO₃ must be dissolved or neutralized by adding acid or acid-forming materials. Sulfuric acid (H₂SO₄) is commonly used to reclaim calcareous soils by increasing nutrient availability (Havlin et al, 2005) Addition of acid-forming NH₄⁺ fertilizers will enhance micronutrient absorption by decreasing soil pH (Brady and Weil, 2004).

Adding salt micronutrients to calcareous soil is generally not effective (Troeh and Thompson, 2005). Iron sulfate ($\text{Fe}(\text{SO}_4)_2$) reacts to form $\text{Fe}(\text{OH})_2$ (an insoluble form). When salts are applied as foliar spraying $\text{Fe}(\text{SO}_4)_2$, $\text{Cu}(\text{SO}_4)_2$ and $\text{Zn}(\text{SO}_4)_2$ they are effective in supply these micronutrients.

Chelate fertilizers are principally in the form of organic and synthetics. Natural or organic chelates include organic and amino acids, ligninosulfonates, lignin polycarboxylates, phenols, humic acids, fulvic acids, flavoniods, and phytosiderophores (Sekhon, 2003). Synthetic chelate fertilizers are formed from micronutrients with Diethylene-triamine penta-acetic acid (DTPA), Ethylene diamine-tetra-acetic acid (EDTA), EDDHA (Ethylene-diamine-di-(o-hydroxyphenylacetic acid) and citric acid (considered a natural chelate). Synthetic chelates are considered more in acid soils than in alkaline soils and are strong complexes. EDDHA will strongly complex Fe over the entire pH range. DTPA can be used for soil $\text{pH} < 7.5$ and EDTA with soils $\text{pH} < 5$. Copper chelates are in the form Na_2CuEDTA . Zinc chelates include Na_2ZnEDTA and ZnDTPA (Havlin et al., 2005).

Alternate wet and dry irrigation

Micronutrient toxicities and deficiencies are affected by management strategies. Micronutrient availability is different in irrigation regimes and with the addition of amendments.

Traditionally, flooded wetland crops such as rice do not receive continuous flooding. Farmers throughout the world, in either naturally rainfed cropping systems, or irrigated systems vary their water regimes to optimize water efficiency or yield parameters. Alternate wet and dry, partial submergence, delayed flooding, and early drainage all comprise irrigation regimes practiced by farmers.

A series of experiments conducted by Bhuiyan and Tuong (1995) demonstrated that continuous standing depth of water throughout the season is not necessary for high rice yields. They showed a savings of about 40–45 percent of the water by applying water in reduced saturated quantities without sacrificing rice yields.

In rice, wetting and drying enhances grain filling period and rice grain quality. A study conducted in China on cultivars Wuyujing 3 (japonica) and Yangdao 6 (indica) were subjected to sufficient irrigation (control) and moderate alternate wet and dry irrigation, and a third alternate severe drought wet and dry irrigation. Compared to the control, the moderate wet and dry irrigation improved grain filled percentage, grain weight, peak viscosity, and mill quality, and less chalkiness. The sucrose synthase (SuS), adenine diphosphoglucose pyrophosphorylase (AGP), starch synthase, and starch branching enzyme were enhanced with moderate wet and dry irrigation, but reduced by the severe wet and dry treatment (JianChang, et al., 2005).

The Lundberg Family Farm practices intermittent irrigation system to irrigate during stand establishment. The Lundbergs adjust the water level to different stages of water, carefully monitoring the correct depth during tillering and maturing. Water metering on the Lundberg Farm helps conserve water. The fields are allowed to dry naturally with correct timing to prevent chalkiness in grains. Water temperature is also monitoring, maintaining water in warming basins before water is allowed to flow in the field (Anon, 1989)

In the Philippines, Tabbal, et al. (2002) found intermittent irrigation increased water productivity by 45% but at the expense of yield. Wet-direct seeding and intermittent irrigation increased yield by up to 17% and increased water productivity.

They found that maintaining ground-water levels allowed greater yield but lower water productivity (Figure 2.1).



Figure 2.1. Mekong Delta in Vietnam. Research is conducted on alternate wet and dry treatments collaboratively by the Water-Saving Work Group of the Irrigated Rice Research Consortium (IRRC) with Vietnam's Plant Protection Department (PPD). The Delta area grows upland rice due to periods of unavailable and insufficient irrigation water. Permission granted by Dr. Bas Bouman through Trina Mendoza, both of the International Rice Research Institute.

Influence of Organic Matter

In submerged soils, Sims and Patrick (1978) found the amounts of element-organic matter complexation were ordered: $Mn < Fe < Cu < Zn$. Bloom (1981) stated the affinity for divalent ions for humic acids and peat were

$Cu > Pb >> Fe > Ni = Co = Zn > Mn = Ca$. The ability of humic and fulvic acids to complex with metals is due to their high oxygen-containing functional groups such as the carboxyls, phenols and carbonyls (Adriano, 1986).

Organic matter in soils includes nonhumic substances and humic substances. Nonhumic substances include carbohydrates, amino acids, proteins, peptides, fats, waxes, resins, organic acids, alkanes, organic bases, and lignin (Dixon and Shulze, 2002). Humic

substances include humic acids, humin and fulvic acids. They range from black-brown colors of humic acid and humin to light brown colors of fulvic acid.

Organic amendments must be used with care in order to avoid toxic effects of organic acids. Organic acids can accumulate in paddy soils, especially at low temperatures. Soil pH influences dissociation of acids, as illustrated with the following formula: $\text{CH}_3\text{COOH} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{H}^+$. The pK_a of this equation is 4.76 when the concentrations of dissociated form (CH_3COO^-) and CH_3COOH are equal. The dissociated form exists mainly at high pH and undissociated is mostly present in the low pH. The undissociated form of organic acids at low pH is toxic to rice roots (Yoshida, 1981).

Rotational cropping with legumes is considered beneficial to nitrogen buildup in the soil. Legumes utilize bacteria to fix atmospheric nitrogen. Mishra et al. (2006) found green manure applied as *Sesbania*, *Leucaena*, cowpea and mungbean residue provided higher yields and greater capacity to supply Zn and Cu compared to wheat straw and farm yard manures. In California, sustainable farming practitioners disc legume straw residue into the soil (Figure 2.2 and 2.3).



Figure 2.2. The Lundberg farmers mow legume cover crop after egg hunt. After mowing, the crop is disked. Photo by Eric Lundberg. Permission granted by the Lundberg Family Farm



Figure 2.3. The Lundberg Farmers discing the copped cover crop. This incorporates legume straw residues for nitrogen fixation. Photo by Eric Lundberg (2009)



Figure 2.3 A second disking by Lundberg farmers.
Photo by Eric Lundberg (2009).
Photo permission granted by the Lundberg Family Farm

CHAPTER THREE

METHODS AND MATERIALS

Greenhouse methods and experimental design

A greenhouse exploratory pot experiment was first conducted in the Cal Poly Earth and Soil Science Department to measure rice and legume, sesbania (*Sesbania macrocarpa*, Muhl.) and faba bean (*Vicia faba* L.) growth in soil (5.71 pH), a sandy loam soil, and Zaca soil, a calcareous soil (8.3 pH). The soil was classified by NRCS as a fine, smectitic [mixed], and thermic Xeric Argialbolls soil collected from Cal Poly State University in the Mission Avocado area., and Zaca soil, a fine, smectitic, thermic Vertic Haploxerolls. Throughout the experiment, one-half of all rice plants and one-half of all legume plants were submerged in 5 gallon buckets of water continuously. The remaining half of the legume plants and the rice plants were not submerged, but watered daily to field-capacity. Rice yield was harvested after 8 weeks at mid-tillering stage and weighted to 0.01 grams.

A second greenhouse pot experiment using loam soil at the same location was conducted to determine micronutrient content rice and legume residue under different water regimes. Dry mass and micronutrient content were examined in rice and rice grown with sesbania from the exploratory experiment incorporated into the soil. The soil was sieved with a 2 mm (USDA No. 10) sieve and 2.8 kilograms each were placed in 24 pots.

Rice (*Oryza sativa* L.) variety S102 was sown in each pot in the Cal Poly Earth and Soil Science greenhouse on May 18, 2008. Seeds were soaked overnight in DI water before sowing. Seeds intended for the flood and AWD treatments were placed on the soil surface in pots filled with water at soil saturation level to simulate rice seed planting by airplane. The greenhouse temperature was set at 22°C. Seedlings were thinned to two

plants per pot (a hill). Plants in eight pots were watered daily to field capacity, or well-drained (drain); 8 received alternate wet-and-dry irrigation (AWD) where pots were submerged for three weeks in 5 gallon buckets of water, drained for one week, and re-submerged for three weeks in the same buckets; and 8 were continuously flooded in the buckets the entire duration of the experiment (flood). Throughout the experiment, rice stems in pots submerged in buckets were covered with water to 5 cm above the stem line. Water in the buckets received 15 mL/L H_2SO_4 due to high pH of the greenhouse water.

In half the pots, green manure was incorporated into the soil. The green manure amendment consisted of *Sesbania macrocarpa* Muhl, common name sesbania, from the exploratory experiment. Sesbania roots and stems were dried, ground, and incorporated into the pots, and homogenized by mixing thoroughly throughout the soil in the pot. Eleven grams were incorporated into each of 12 of the pots.

The growing area was in a north-south facing greenhouse on the two middle benches of the south most side. The area 3.2 meters wide and 2.7 meters long and contained air vents, a heater, but no cooling system (Figure 3.1).

Each treatment was replicated four times in a blocked arrangement. Treatments were randomized and blocked. Drain pots were placed between buckets for protection (Figure 3.1).



Figure 3.1. Rice plants grown in pots in the Cal Poly Earth and Soil Science greenhouse.

After 3 weeks submergence in the buckets of water, pots receiving the AWD treatment were placed above the bucket to dry for 1 week. This cycle was repeated throughout the growing period. Water applied to the buckets was brought to an approximate pH of 6.5 with 15 ml/L H_2SO_4 due to the high pH of the greenhouse water.

North to South	Row East East	Row East West	Row West East	Row West West
	Block 1		Block 3	
	Flood	AWD sesbania	Flood sesbania	AWD sesbania
	Drained	Drained sesbania	Drained	Drain sesbania
	AWD	Flood sesbania	AWD	Flood
	Block 2		Block 4	
	Flood	AWD sesbania	Flood sesbania	Flood
	Drained sesbania	Drained	Drain sesbania	Drain
	Flood sesbania	AWD	AWD	AWD sesbania

Figure 3.2. Map of rice grown in a greenhouse pot experiment. Pots were placed in a split plot design with two factors, water and sesbania as organic matter incorporated into the soil.

All pots received 0.8 g (150 ppm) KCl at the beginning of each AWD cycle at the time of re-submergence, and 1.00 grams (150 ppm) urea in split applications at the beginning of tillering and at end of tillering.

After 16 weeks plants were harvested. Stems were cut 5 cm above the soil line to avoid contamination. Grains were removed from panicles and dehusked by hand with Al oxide sand paper. Stems and grain were placed in an oven at 180 °F for 48 hours. Dry matter weight was recorded in grams (0.01).

Analytical methods

The pH measurements were read on an accumet ab15 soil pH meter (Fisher Scientific). Readings of the virgin soil was recorded, and recorded from mid-tillering stage and at three week time intervals through the remaining time of the experiment. End harvest pH readings were taken through standing water to soil level in each pot, including drain treatment where water was placed above the soil line at the time of each reading.

Soil micronutrients were measured using the DTPA (Diethylene triamine pentaacetic acid), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, (buffered at pH 7.3) method of extracting micronutrients. The DTPA solution is a chelate solution is a solution containing 0.005 M DTPA with 0.1 M TEA (triethanolamine) and 0.01 M dihydrate calcium chloride adjusted to pH 7.3 by 1.0 N HCl. Twenty grams of the solution was mixed with 10 grams of soil (2:1) and shaken for 2 hours. Solution was filtered through a No. 42 Whatman filter paper (Lindsay and Norvell, 1978, Page et al., 1982).

Plant samples were prepared for acid digestion by a modified EPA 3050B method (U.S. EPA, 1996). Samples of 0.300 ± 0.005 were placed in a clean, acid washed 150 ml beaker. Each beaker received 15 ml nitric acid. Beakers were covered with a ribbed watch glass (65 mm) and placed in a heating element at 55 °C for 12 hours (overnight).

After preparation, both soil and stem samples were analyzed for Fe, Mn, Zn, and Cu using the Earth Science and Soil Science Department Atomic Absorption Spectrophotometer Varian Spectra AA model number 55B at the wavelengths specified for the metal micronutrients. The physical properties of the soil are given. The soil cation exchange capacity was obtained through addition of cations (Table 3.1).

Table 3.1. Physical and chemical properties of the Concepcion sandy loam soil.

Characteristics	Value	Characteristic	Value
Sand %	54	Fe ppm	96
Silt %	19	Mn ppm	28

Clay %	27	Cu ppm	2.9
pH (1:2)	5.71	Zn ppm	6.0
Eh	178	Ca ppm	2135
Color (dry)	10YR 4/3	Mg ppm	760
Color (moist)	10YR 3/2	K ppm	32
Organic Matter %	3.51	Na ppm	50
N %	0.185	H+	5.4
C:N ratio	9.45	CEC	23

Statistical Methods

A Minitab version 15 general linear model (GLM) factorial (3 water treatments levels X 2 sesbania treatment levels) ANOVA procedure was used to compare treatments for all soil pH, soil micronutrient content, stem micronutrient content, and yield data at the 5% significance level.

Tukeys HSD pairwise comparison was used to separate significant simple effects at the 5% significant levels.

CHAPTER 4

RESULTS AND DISCUSSION

Exploratory experiment yield

In Asian countries, sesbania is grown as a rotational crop to enhance soil and plant bioavailability of nutrients and micronutrients. The effectiveness in providing nitrogen to lowland paddy rice with *Sesbania rostrata* intercropped in a typic Tropaquept soil in Sierra Leone was demonstrated by Bar et al. (2000). In many states in the U.S., sesbania is considered invasive (USDA NRCS, 2009). Legumes such as faba beans are therefore rotated for nutrient sources. Faba bean is a temperate climate crop originating from the Mediterranean, making California's climate ideal for rotating faba bean as a cool season crop preceding rice planting.

The exploratory experiment demonstrated the effects of water and soil type on rice and legume growth. Faba bean yield did not differ in either Zaca clay or Concepcion loam soil but was higher in drain conditions, with only one plant surviving in the flood treatment. Yield of both rice and sesbania were higher in flood water treatment in Concepcion soil (Table 4.1) The sesbania was selected for a legume to simulate a compatible cropping system for rice in the subsequent experiment (Figure 4.1) .

Table 4.1. The effect of two water levels and soil types on yield in rice and legumes

	rice (grams)	sesbania (grams)	faba beans (grams)
drain	8.5 ± 1.7	13 ± 3.6	4.3 ± 0.67
flood	25 ± 4.3	39 ± 7.2	0.22 ± 0.00
Zaca drained	1.4 ± 0.43	4.8 ± 1.8	4.8 ± 0.72
Zaca flood	3.6 ± 0.69	10 ± 1.3	3.3 ± 0.83

Experiment on effect of water on micronutrient content and yield in rice

Observations

Plants appeared different in color and habit among water treatments. Drained rice plants appeared green and showed no symptoms of chlorosis throughout the tillering

stages. Plants appeared to have more tillers and be taller. After advanced tillering stage, rice plants grown in drained slowed growth processes in height, but remained green (Figure 4.1). Plants grown under AWD and flood conditions were not as green appearing as plants grown under the drain treatment (Figure 4.1), and those grown in pots under AWD and flooded conditions were without green color in the bottom one-third of the plant length (Figure 4.1).

Rice plants grown in continuous flooded soils and alternate wet and dry soils (in 5 gallon buckets) appeared less green but taller (Figure 4.1). Necrosis occurred in one plant in a pot with alternate wet and dry flooded soil (with sesbania incorporated). One plant each in two pots in continuous flooded soils (one with sesbania incorporated and one without sesbania incorporated) developed signs of stem rot disease symptoms and were removed from their respective pots. Thus, in 3 of 24 pots, 1 plant remained through harvest.

Adventitious roots grew in plants grown in continuous flooded pots. In rice plants grown in alternate wet and dry flooded pots, adventitious were sparse to non-existent.

The sandy loam soil became gleyed (color was 2.5 Y on the Munsen color chart) in both the continuous flooded soil and alternate wet and dry flooded soil. Algae presence was noted, and growth was controlled by addition of H_2SO_4 to irrigation water to neutralize the pH of the water.

The rice plant in pots with incorporated sesbania residue initially had a strong organic smell. The smell subsided after the first 2 weeks under submergence. All plants in pots under submergence, both under continuous flooding and alternate wet and dry flooding, developed a sulfur smell.

Tiny bubbles appeared in buckets both in the continuous flooded pots and alternate wet and dry flooded pots. Although water was added to buckets daily to maintain a level approximately 10 cm from the top, the water was in a stagnant state throughout the growing period. Water temperature was not recorded. Excessively low water temperature in cold ambient temperatures may cause sterility.

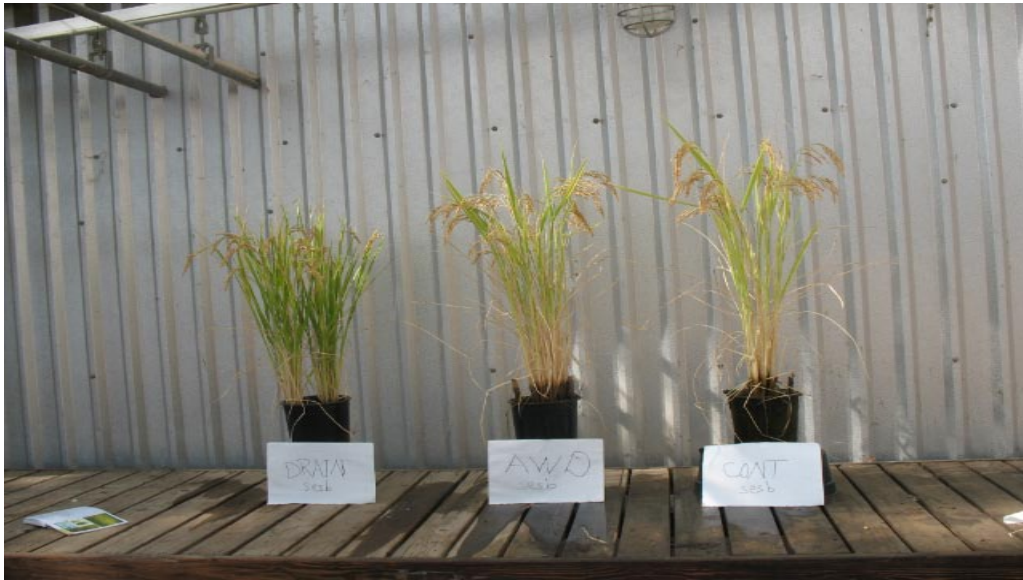


Figure 4.1. Growth comparison of rice grown under three different water treatments. Treatments from left to right: drain, AWD, and flood

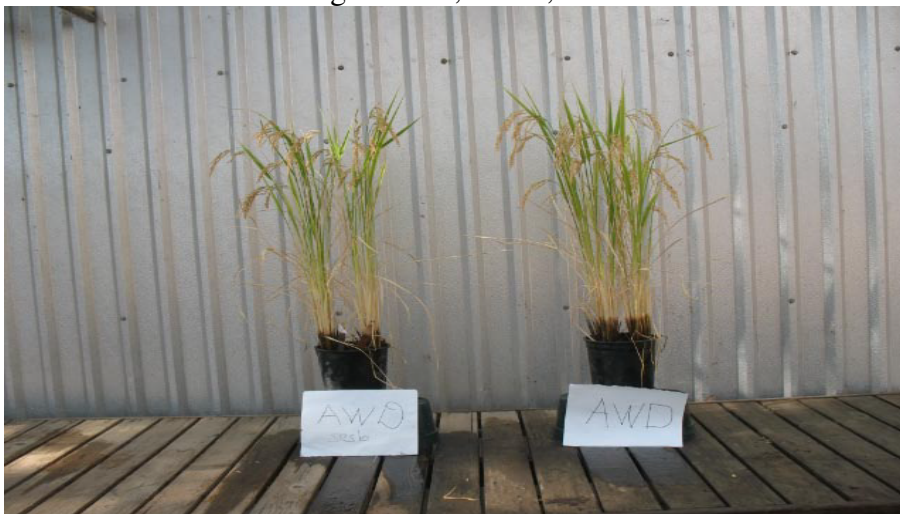


Figure 4.2. Growth comparison of rice grown under two different OM matter treatments. Sesbania incorporated into the soil (pot on the left) and without sesbania incorporated into the soil (pot on the right) were similar in height and habit.

Soil pH

All soil pH readings were higher than the original 5.71. Soil pH neutralizes under submerged conditions. When soils are first submerged, soil pH may decrease due to CO₂ formed in aerobic respiration by bacteria (Ponnamperuma, 1972). The subsequent increase in pH over time is due to consumption of H⁺ ions and to the presence of hydroxides of Fe (II), Mn (II), and of NH₃, or increase in OH⁻ ions. The CO₂ accumulates; decreasing pH of alkaline soils and slows the increase in pH of acid soils (Kirk, 2004). As a result the pH values of all submerged soils tend to stabilize at a range between pH 6.5-7 (Kirk, 2004). After the first few weeks of submergence, pH stabilizes at 6.72-7.2 (Ponnamperuma, 1972).

The average soil pH for all treatments declined with duration of time in water (Figure 4.3). The highest average soil pH (6.93 ± 0.024) occurred in the drained treatment with no sesbania added to the soil. The lowest average soil pH, 6.75 ± 0.030 , occurred in continuously flooded water treatment with sesbania incorporated into the soil (Appendix A). Both drain sesbania treatment and drain no sesbania treatment were significantly higher than flood sesbania treatment.

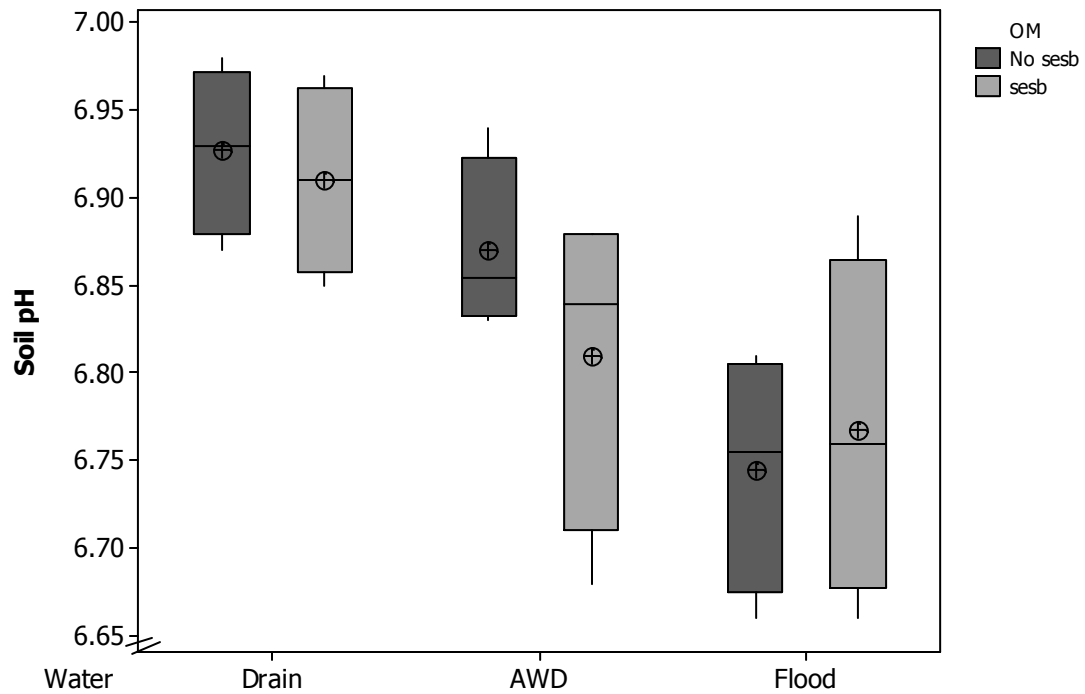


Figure 4.3. Boxplot of soil pH values of rice grown in a greenhouse pot experiment. The chart displays whiskers and stems. The bottom-most point of the whisker is the lowest value, the bottom line of the box is the first quartile (25 percentile), the second line on the box is of the median quartile (50 percentile), the third line on the box is the third quartile (75 percentile), and the top most point on the top whisker is the highest value. Encircled star indicates the median.

DTPA extractable soil micronutrient concentrations

Average Fe soil concentration was more than two times lower (57 ± 4.2 and 55 ± 4.2 , no sesbania and sesbania respectively) in drain than in flood treatments (130 ± 11 for no sesbania and 117 ± 6.6 for sesbania) (Figure 4.4). Iron toxicity occurs in lowland soils in acid sandy soils and in some peat soils (Yoshida, 1981). The duration of submergence also influences iron soil content. Yoshida (1981) reported iron soil content as high as 300 ppm after 4 weeks submergence, then decreasing exponentially to 50-100 ppm at six months submergence where concentration remained steady. Critical levels of Fe deficiency in soil are $<4-5$ ppm, DTPA- CaCl_2 , pH 7.3 (IRRI 2002). The original DTPA extractable Fe content was 96 ppm.

Water and OM were significant predictors of soil Mn concentration. A significant ($p < 0.05$) interaction occurred between water and OM (sesbania). The original DTPA Mn soil content was 28 ppm. The average Mn soil concentration was lower, in the drained water treatments than in the AWD or flood treatments. The flood treatment with sesbania was higher (143 ± 6.9 ppm) than any treatment, including the flood treatment with no sesbania incorporated into the soil (89 ± 8.1 ppm). The average Mn. The original DTPA Mn soil content was 28. The high soil content in the flood sesbania treatment may relate to the original amount of Mn in the ground sesbania under continuous submerged conditions.

It is interesting to observe both Mn and Zn increased in the flood water treatment with sesbania added. DTPA extractable Neue et al.(1998) observed higher Mn with Zn added to waterlogged soil, while the uptake and translocation of Cu, Fe, and P were lower. Further studies are needed to analyze different soil amendment levels of micronutrients and macronutrients, particularly P, Mg. Another possible reason for elevated Mn soil concentration may be that the original ground sesbania from exploratory experiment possibly contained a high amount of Mn, and under continuous submerged conditions was released. The ground sesbania was not analyzed, a procedural mistake of the experiment.

The DTPA Cu soil concentrations rose sharply from drain water treatment to the AWD and to the flood treatment. The average soil Cu concentration in drain soil, 1.62 ± 0.024 in the no sesbania treatments, and 1.61 ± 0.017 ppm in the sesbania treatment, rose to 3.97 ± 0.12 ppm and 4.37 ± 0.23 ppm in the AWD water treatments for no sesbania and sesbania treatments, to 5.00 ± 0.023 ppm and 4.85 ± 0.15 ppm in the flood treatments

with no sesbania and sesbania treatments respectively (Figure 4.4). The original DTPA soil Cu content is 2.93 ppm. Although soil Cu concentration in the drain concentration was low, it was above the critical level, 0.2-0.3 ppm, DTPA + CaCl₂, pH 7.3 (IRRI, 2002). Factors possibly contributing to the low soil Cu content in the drain treatment include a sandy loam soil, higher pH in the drain treatment, and a possible Zn-Cu competition since Zn was highest in the drain treatment (Figure 4.4) (Smith, 2007).

An opposite trend to Fe, Mn and Cu was observed in the DTPA extractable Zn soil content among all water treatments. The average DTPA extractable soil Zn concentrations in the drain water treatment were significantly higher (13.5 ± 0.48 ppm and 12.6 ± 0.72 ppm respectively in no sesbania and sesbania treatments) than in AWD (5.6 ± 0.37 ppm and 5.8 ± 0.30 ppm respectively in the no sesbania and sesbania treatments) and flood treatments (7.2 ± 0.47 ppm and 9.1 ± 0.71 ppm respectively in no sesbania and sesbania) (Figure 4.4). No significant difference was found in sesbania groups within water treatments (Figure 4.4). Soil Zn content was significantly higher in flood treatment with sesbania added where soil pH was the lowest. The DTPA extractable Zn content for the virgin soil is 6.00 ppm.

High soil Zn content may affect growth. Rattan and Shukla (1984) reported lower dry matter yields in paddy rice from 8.37 grams to 3.97 grams as DTPA extractable Zn soil content rose from 7.53 ppm to 15.8 ppm, and further decreasing to 0.96 grams as Zn soil content rose to 36.7 ppm.

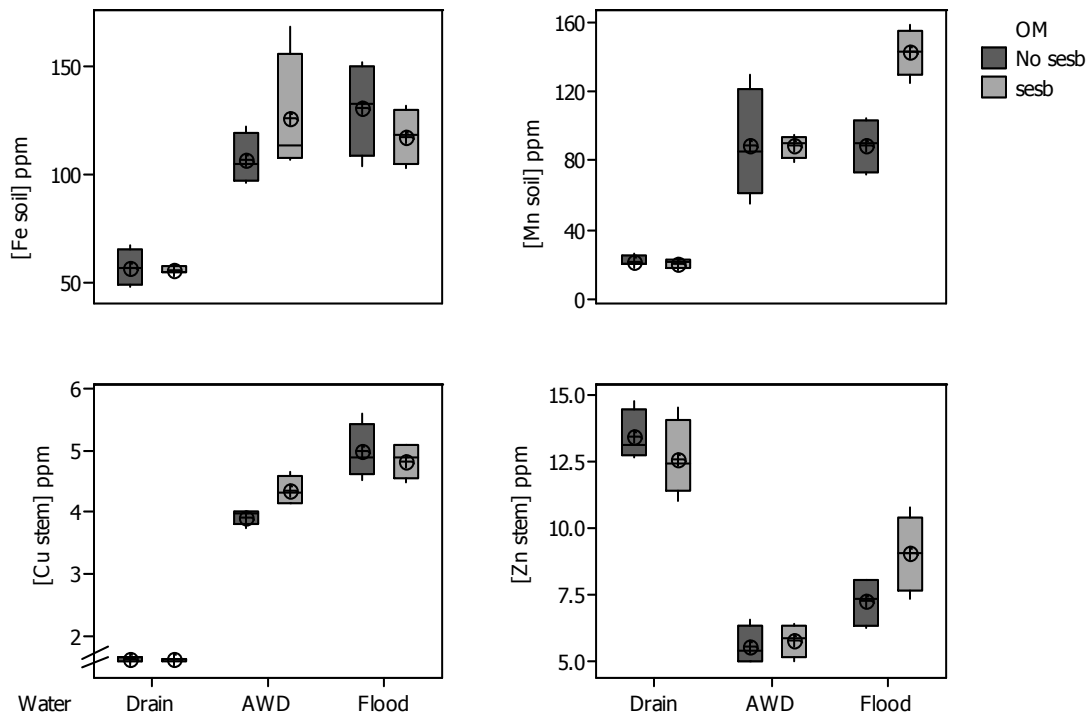


Figure 4.4. DPTA extractable soil metal micronutrients in greenhouse pots.

Stem micronutrient concentrations

Whole plant analysis was conducted in a Cal Poly Earth and Soil Science Department greenhouse pot study on rice plants in 24 pots in a randomized split plot. Each pot contained two plants per hill. At harvest, all but 3 pots still retained the full hill. Plants in pots with sesbania incorporated into the soil appeared slightly stunted in vegetative growth (Figure 4.2)

Stem concentrations were compared with agricultural industry critical values. A critical value is defined as the concentration of an essential element at which there is a 5–10% reduction in growth or yield. Critical sufficiency for nutrient concentration in rice plants based on Y leaf concentrations for Fe stem concentration is 90-190 ppm, for Mn,

40-740 ppm, for Zn 20-160 ppm and for Cu 6-25 ppm. This information is derived from Counce and Wells study (1986) on nutrient amounts in varieties of rice.

Iron concentrations among water groups did not vary significantly. The highest average Fe stem concentration (183 ± 2.4 ppm) was in the flooded group with sesbania incorporated into the soil, and the lowest was in the flooded group with no sesbania incorporated into the soil (130 ± 8.9 ppm) (Figure 4.5). No sesbania effect was observed. Rice stem toxicity occurs when concentrations exceed 300 ppm (Dobermann and Fairhurst, 2002).

The average Mn stems concentrations for whole plant rice was critically high (921 ± 7.4 ppm) in the AWD group without sesbania added (Figure 4.5) (Counce and Wells, 1986). Stem Mn concentration in the drain water treatment differed significantly from the AWD and the flood water treatments ($p < 0.05$) but not within the groups with sesbania incorporated into the soil (Figure 4.4). The treatment group with the lowest average Mn stem concentration (216 ± 2.9 ppm) was the drain water group without sesbania incorporated into the soil. Manganese toxicity in paddy rice is rare. When it occurs, it is on acid soils $< \text{pH } 5.5$, or soils with high amounts of reducible Mn. It can occur when uptake mechanisms for tolerance are poor. The mechanisms involve root to shoot ratio and organic acid balance such as accumulation of phenolic acids; and imbalance of K, Si, P, Ca, and Mg (Dobermann and Fairhurst, 2002). These subjects are recommended for future research.

Cu stem concentration was analyzed in water groups and sesbania groups. The Cu stem concentration was constant among water groups (Figure 4.5). Addition of sesbania did not affect Cu stem concentration within drain and AWD water groups (Figure 4.5). The Cu concentration for all treatments was within the sufficiency range for all treatment

cited by Counce and Wells (1986) (Figure 4.5). Copper binds with organic ligands in the soil and in the xylem sap in plants (Wilkinson, 2000). Liu et al, (2008) found varietal difference in known Cu tolerant and sensitive varieties with additional amendment of Cu fertilizer.

Significant Zn differences were observed among water group. The Zn concentration was more than 5 times higher in drain treatment (138 ± 6.6 ppm in drain water with *Sesbania* added) than in both AWD and flood (27 ± 1.3 ppm in flood water treatment with *Sesbania* added).. No difference was observed in *Sesbania* within water groups.

It is interesting to note the highest Mn stem concentrations corresponded to the lowest Zn stem concentrations, both in the AWD water treatment (Figure 4.5). A minitab correlation statistical analysis revealed a strong negative pearson correlation and significant difference ($r=-0.841$, $p=0.000$) between Mn and Zn. Sajwan and Lindsay (1986) found that Zn deficiency observed in flooded paddy soils can be explained in part by solubilization of Fe and Mn creating an antagonistic effect on the availability and uptake of Zn. They hypothesized Fe dissolves at reduced microsities and precipitates again as ferrosic hydroxide ($\text{Fe}_3[\text{OH}]_8$) in zones of higher oxidation, facilitating a depressed Zn^{2+} due to Fe solubilization. Neue et al., (1998) observed a higher uptake and translocation of Fe, Mn, and Cu when uptake and translocation of Zn was low.

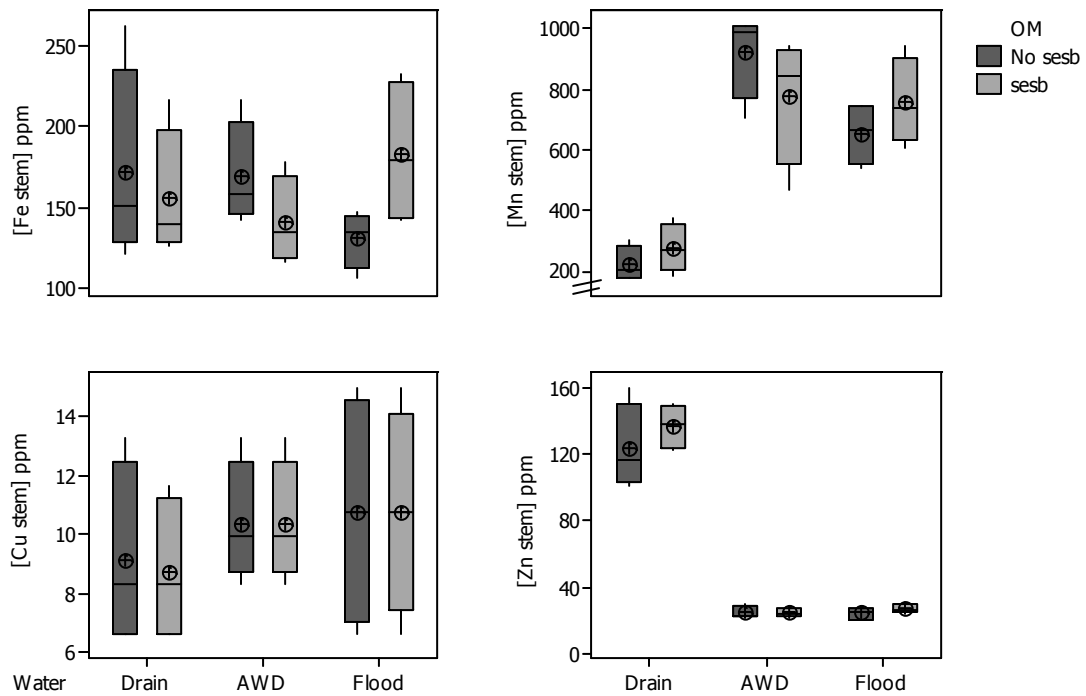


Figure 4.5. Stem micronutrients concentrations (ppm) in a rice pot experiment.

Yield

Yield was measured as the dry plant weight per pot. Only dark or black grain was discarded. Grain weight was reported to 0.01 grams. The grain weight in drain water treatment, 21 ± 2.3 and 20 ± 0.324 grams respectively in the no sesbania and sesbania groups), was significantly lower than the weight in the AWD groups, 32 ± 1.2 and 30 ± 3.9 grams respectively in the two sesbania groups, and the grain weight in the flood water treatments, 29 ± 3.4 grams and 334 ± 2.1 grams respectively in the sesbania groups (Figure 4.6). Converting grains per pot to tons per hectare, this amount for the lowest drain treatment is 2.11 ± 0.235 and 2.02 ± 0.032 tons per hectare respectively in the no sesbania and sesbania group, 3.27 ± 0.125 and 3.01 ± 0.390 tons per hectare respectively in the two sesbania groups for the AWD water groups, and 2.94 ± 0.343 and 3.44 ± 0.214 tons per hectare respectively in the sesbania groups for the flood water group. In 2008

the global yield was 4.25 tons per hectare. In the U.S. in the same year, the amount was 7.68 tons per hectare (IRRI, 2008). California rice yield for 2008 was 10.3 tons per hectare (USDA NASS, 2009).

No significant difference in grain yield was found among the AWD and flood water treatments. Timing and application of water in irrigated paddy fields is important. Yoshida (1981) noted that reduced water use during the reproductive stage can affect yield by lowering sterility. Crop water deficit at vegetative stage can reduce tillering, leaf area, and grain yields if water is not reapplied with adequate recovery time before flowering (Castillo et al. 1992).

Sesbania incorporated into the soil had no significant affect on grain weight within the water treatments. Only one level of sesbania, 11 grams (1.1 tons per hectare), was applied to each pot. A more thorough investigation of additional levels of organic matter amended into the soil and different kinds of organic matter is needed to determine the effect of organic matter on rice grain yield.

Stem weight decreased from drain treatment to AWD and flood treatment. The two lowest stem weights, 18 grams (yield equivalent, 1.83 tons per hectare) and 19 grams (yield equivalent 1.86 tons per hectare), were observed in AWD and flood treatments, and were due to each experiencing a loss of one plant per pot. Average stem weight was highest in the drain water group, 37 ± 1.8 grams and 36 ± 2.7 grams (yield equivalent, 3.7 ± 0.18 tons per hectare and 3.6 ± 0.27 tons per hectare) respectively in the no sesbania and sesbania groups, and lowest in the flood group, 26 ± 3.6 grams and 32 ± 1.0 grams (yield equivalent, 2.6 ± 0.37 tons per hectare and 3.2 ± 0.10 tons per hectare) respectively in the no sesbania and sesbania groups.

The ratio of the yield of grain to stem was compared among treatments. Grain to stem yield ratio was higher in the AWD and flood treatments than in the drain groups (Figure 4.6). Grain to stem ratio for both AWD and flood water treatments were similar. Grain to stem ratio did not differ among sesbania treatments (Figure 4.6)

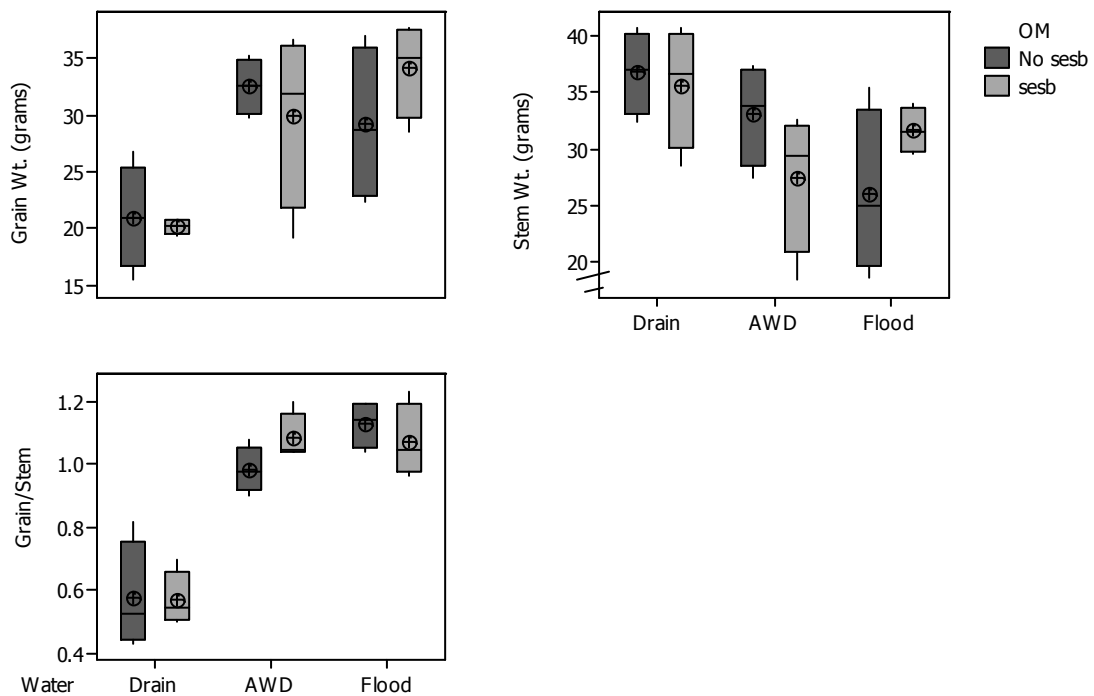


Figure 4.6. Grain weight, stem weight, and grain to stem weight ratio in rice grown in pots in loam soil.

Comparison of grain and stem micronutrient content

Grain yield and stem micronutrient content at the water level were compared. Since sesbania groups were highly not significant within water groups, they were omitted in the ANOVA comparison. Grain weight was significantly different from Fe stem content within the three water groups, with a strong interaction occurring between Fe stem concentration and water (Appendix D). Average stem content was highest in the flood treatment with sesbania added (Figure 4.5), the treatment with the highest median grain weight (Figure 4.6). Average Fe stem content was higher than the optimum rice

sufficiency range listed at 60-100 ppm by Doberman and Fairhurst (2002) but lower than the toxicity range (over 300 ppm). Iron stem content involves several factors, including iron excluding power (Yoshida, 1981) of rice roots. Further studies on root to shoot ratio are encouraged.

No significant difference occurred between Mn content and grain content nor did a Mn-water interaction occur (Appendix D). Manganese was the highest and at toxic levels (>800 ppm) (Doberman and Fairhurst, 2002), in the AWD water groups with no sesbania added but lower in the same water group with sesbania added (Figure 4.5). Grain weights in the AWD group with no sesbania and sesbania added were reversed in amounts: lower in the AWD with no sesbania added group than in the same group with sesbania added (Figure 4.6). Rice varieties vary in tolerance to Mn toxicity. Wang et al. (2002) observed a difference in tolerances to Mn toxicity in japonica rice variety Azucena and indica rice variety IR1552. They measured and rated tolerance by toxicity symptoms. They found the shoot concentrations in IRI552 were 2 to 5 times higher than in Azucena, with no brown spots, the main Mn toxicity symptom, observed in IRI552.

Grain weight significantly differed from Cu stem concentration. Water was not a significant predictor, nor did a Cu-water interaction occur. Average Cu stem concentration were lower in the drain water treatments than in the AWD and to flood treatments (Figure 4.5), the two water groups with higher grain yields (Figure 4.6). Copper stem concentrations were within the optimum rice sufficiency level of 7-15 ppm listed by Doberman and Fairhurst (2002).

Grain weight significantly differed from Zn stem concentration between water groups (Appendix D). A significant interaction occurred between Zn stem concentration and water (Appendix D). Grain weight in drain treatment was lower than in the AWD or

flood treatments Content in the drain treatment approached toxic content reported by Chino (1981) when levels are between 100-300 ppm (Doberman and Fairhurst, 2002). Plants and varieties vary widely in their ability to tolerate Zn toxicity. Research conducted by Dong et al. (2004) observed tolerance levels differences in quantitative traits locis in lines of rice. Bronzing was used as an indicator of zinc tolerance and rated from 0 to 10. They found no effect on growth until 600 ppm Zn^{2+} concentration. Zinc stem concentrations were within the optimum range listed by Dobermann and Fairhurst (2002) in the AWD and flood water treatments where grain weight was highest.

CHAPTER 5

SUMMARY AND CONCLUSION

Summary of the Experiment

As the world faces an increasing shortage of water, research in rice cropping systems is focused on water saving techniques. A wide range of techniques from varietal improvements to timing of flooding are currently undertaken to boost global rice production.

A greenhouse pot experiment was conducted in the California State Polytechnic University Earth and Soil Sciences greenhouse to determine soil and stem micronutrient content, and affects on yield in rice grown in three different water regimes, well drained (drain), AWD, and continuously flooded (flood), with half the pots receiving sesbania incorporated into the soil. Statistical analysis was performed using Minitab 15.

Soil pH decreased from drain to AWD and flood treatment. Soil pH first rises in submerged soil, then neutralizes. Soil pH was measured at each AWD cycle, but only analyzed at the end of the experiment to coincide with micronutrient concentrations.

Differences in water treatments were demonstrated in all metal micronutrient DPTA extractable soil. The most notable soil concentration difference occurred in DPTA extractable Zn which decreased markedly under AWD and flood conditions.

No differences in water treatments were observed among average Fe and Cu stem concentration. Manganese stem concentration increased with AWD and flood treatments while Zn stem concentration decreased sharply. Manganese toxicity can occur in rice and can be alleviated by silica slags. Zinc deficiency in rice conditions is a worldwide problem affecting the diet of billions of humans dependent upon Zn for their staple food. Foliar applied Zn can boost Zn concentration. Chavan and Banerjee (1979) reported increased amounts of Fe decreased the level of Fe in rice stems. It is interesting to note

that Zn stem concentration in the drain treatment was the toxic range, possibly affecting grain yield in the drain treatment.

Concentration analysis occurred at the end of the experiment. Concentration over time and in lower, middle, and panicle sections of the rice plant provide a clearer picture of uptake and translocation of nutrients in a plant. At eight weeks, the plants in the drain pot treatments looked green and healthy.

Yield was appreciably affected by water treatments. Grain weight increased with increasing water; stem to grain ratio increased from drain to AWD and drain to flood treatments. No significant differences were detected between average AWD and flood treatments. As the rice plants aged throughout the duration of the experiment, the leaves became the micronutrient source and the grain became the sink (Yoshida, 1981).

The Fe and Mn stem concentration differences were not as robust as desired due to fewer replicates than needed. Adding two more replicates to each treatment would provide larger degree of freedom, and greater confidence in determining the effects of the water and OM. Since the atomic absorption analysis was set skillfully accurate without need for dilutions by the Earth and Soil Science department technician, Craig Stubler, larger variations were due to inadequate replicates and sample sizes.

Research Recommendations

Macronutrients should be included to develop macronutrient to micronutrient ratios and to attain better understanding of nutrients availability through concepts of mobilization of nutrients. Macronutrient to micronutrient ratios developed through DRIS aid in understanding lack of nutrient availabilities. Nutrient balance and water savings and thus global food security can be more accurately achieved through better understanding of complete nutrient analysis in rice plant cropping systems.

A two-year field study to accompany a greenhouse study would boost results. Field research provides more realistic data, as it is usually conducted at the site of the cropping system.

Efforts are needed to inspire and encourage youth to study rice. A program in Arkansas, “Rice for Kids”, was started by two farmers who donated one acre to local youth for an educational program at Riverview Elementary in Judsonia and Bald Knob Elementary School. Young students learn about the economics and science of rice farming. Similarly, The IRRI offers Rice Camp for teens wishing to learn about science and techniques in cropping rice. Youth at Rice Camp participate in all aspects of farming and laboratory research under top-notch soil and plant scientists (Figure 4.7).



Figure 4.7. IRRI Rice Camp 2006. Permission granted by IRRI Trina Mendoza and Chrisanto Quintana

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APPENDICES
Appendix A.

Table 1. Raw data for rice Fe variables

Water	OM	Soil % OM	Soil pH	Soil Eh	Plant Dry Wt			DTPA Extract Fe	Fe concentration root			Fe Uptake		
					root	stem	grain		stem	grain		root	stem	grain
					--Grams--			micrograms per gram	micrograms per gram			micrograms per pot		
Drain	None	3.00	6.87	74	13	32	26	47	1172	152	55	15243	4905	1467
Drain	None	3.09	6.95	79	18	41	20	67	565	150	53	10356	6129	1090
Drain	None	3.43	6.91	57	14	36	15	58	1455	263	42	20894	9380	638
Drain	None	3.37	6.98	71	16	57	22	54	828	120	17	13055	4595	354
Drain	Add	3.42	6.88	60	18	41	20	54	622	138	42	11395	5652	851
Drain	Add	3.27	6.94	51	13	38	21	54	295	217	40	4042	8324	829
Drain	Add	2.98	6.97	68	11	29	20	54	448	140	48	5071	3997	967
Drain	Add	3.21	6.85	51	14	35	19	57	1013	125	42	14045	4360	801
AWD	None	3.11	6.87	-543	14	27	30	112	2648	157	38	36070	4301	1140
AWD	None	2.92	6.84	-544	15	36	35	96	3375	160	53	50558	5733	1873
AWD	None	2.80	6.94	-546	17	37	34	98	2487	217	43	42671	8100	1470
AWD	None	2.69	6.83	-515	14	32	31	122	2888	142	47	41390	4510	1452
AWD	Add	3.38	6.8	-512	10	33	34	107	2495	128	63	25674	4176	2166
AWD*	Add	3.33	6.68	-546	10	18	19	169	3170	115	48	30305	2094	927
AWD	Add	3.42	6.88	-533	10	30	37	116	2798	178	43	28151	5433	1589
AWD	Add	2.70	6.88	-521	16	28	29	111	2555	140	13	42132	3949	392
Flood	None	3.00	6.66	-669	11	27	32	153	6792	137	38	72399	3705	1241
Flood	None	3.02	6.79	-660	16	35	37	103	6300	147	40	100359	5205	1482
Flood*	None	2.93	6.81	-664	14	19	22	122	7902	105	48	107700	1943	1073
Flood	None	2.92	6.72	-665	12	22	25	143	9163	132	42	107486	2976	1038
Flood*	Add	3.33	6.73	-671	15	33	33	124	9410	145	43	138986	4722	1433
Flood	Add	3.29	6.79	-672	15	30	38	102	10528	233	47	157925	7105	1761
Flood	Add	3.19	6.66	-646	12	29	28	132	9978	142	40	123332	4174	1139
Flood	Add	3.17	6.89	-671	18	34	37	111	9555	213	45	171799	7253	1660

*pots with only one plant. All other pots contained two plants per pot (a hill)

Appendix A (cont)

Table 2. Raw data for rice Mn variables

Water	OM	Soil %OM	Soil pH	Soil Eh	Plant Dry Wt			DPTA Mn Extract	Mn concentration			Mn Uptake		
					root	stem	grain		root	stem	grain	root	stem	grain
					----grams----			Micrograms per gram	--micrograms per gram--			micrograms per pot--		
Drain	None	3.00	6.87	74	13	32	26	20	27	217	25	347	7007	667
Drain	None	3.09	6.95	79	18	41	20	26	18	170	20	336	6946	409
Drain	None	3.43	6.91	57	14	36	15	20	33	298	18	479	10626	281
Drain	None	3.37	6.98	71	16	57	22	21	23	180	17	368	6893	355
Drain	Add	3.42	6.88	60	18	41	20	23	23	182	18	428	7423	375
Drain	Add	3.27	6.94	51	13	38	21	23	10	293	17	137	11270	345
Drain	Add	2.98	6.97	68	11	29	20	19	20	242	22	226	6899	434
Drain	Add	3.21	6.85	51	14	35	19	18	58	373	20	809	13023	385
AWD	None	3.11	6.87	-543	14	27	30	56	35	1002	22	477	27504	645
AWD	None	2.92	6.84	-544	15	36	35	130	55	1012	22	824	36251	761
AWD	None	2.80	6.94	-546	17	37	34	92	47	972	22	801	36324	735
AWD	None	2.69	6.83	-515	14	32	31	79	47	702	27	669	22337	830
AWD	Add	3.38	6.8	-512	10	33	34	95	42	802	27	429	26089	912
AWD*	Add	3.33	6.68	-546	10	18	19	89	62	467	18	590	8497	351
AWD	Add	3.42	6.88	-533	10	30	37	91	50	940	25	503	28640	916
AWD	Add	2.70	6.88	-521	16	28	29	79	45	887	25	742	25007	735
Flood	None	3.00	6.66	-669	11	27	32	104	45	728	35	480	19745	1133
Flood	None	3.02	6.79	-660	16	35	37	101	40	597	22	637	21174	803
Flood*	None	2.93	6.81	-664	14	19	22	72	82	537	30	1113	9932	666
Flood	None	2.92	6.72	-665	12	22	25	79	73	745	28	860	16839	707
Flood*	Add	3.33	6.73	-671	15	33	33	159	83	607	25	1231	19756	827
Flood	Add	3.29	6.79	-672	15	30	38	146	43	703	22	650	21417	817
Flood	Add	3.19	6.66	-646	12	29	28	141	88	943	32	1092	27794	902
Flood	Add	3.17	6.89	-671	18	34	37	125	78	772	25	1408	26235	924

*pots with only one plant. All other pots contained two plants per pot (a hill)

Appendix A. (cont)

Table 3 Raw data for rice Cu variables

Water	OM	Soil %OM	Soil pH	Soil Eh	Plant Dry Wt			DPTA Cu Extract	Cu concentration			Cu Uptake		
					root	stem	grain		root	stem	grain	root	stem	grain
					grams per pot			Micrograms per gram	Micrograms per gram			Milligrams per pot		
Drain	None	3.00	6.87	74	13	32	26	1.56	11	13	5	173	431	133
Drain	None	3.09	6.95	79	18	41	20	1.66	12	10	3	214	408	68
Drain	None	3.43	6.91	57	14	36	15	1.62	12	7	5	167	236	77
Drain	None	3.37	6.98	71	16	57	22	1.66	8	7	3	131	255	71
Drain	Add	3.42	6.88	60	18	41	20	1.62	12	10	5	214	409	102
Drain	Add	3.27	6.94	51	13	38	21	1.56	12	12	5	160	448	104
Drain	Add	2.98	6.97	68	11	29	20	1.64	8	7	5	94	190	100
Drain	Add	3.21	6.85	51	14	35	19	1.60	17	7	5	231	233	96
AWD	None	3.11	6.87	-543	14	27	30	4.00	5	13	3	68	366	99
AWD	None	2.92	6.84	-544	15	36	35	3.96	10	10	3	150	358	117
AWD	None	2.80	6.94	-546	17	37	34	4.02	8	10	3	143	374	113
AWD	None	2.69	6.83	-515	14	32	31	3.76	10	8	3	143	265	104
AWD*	Add	3.38	6.8	-512	10	33	34	4.24	7	13	3	69	434	114
AWD	Add	3.33	6.68	-546	10	18	19	4.66	7	8	2	64	152	32
AWD	Add	3.42	6.88	-533	10	30	37	4.42	5	10	3	50	305	122
AWD	Add	2.70	6.88	-521	16	28	29	4.14	7	10	2	110	282	49
Flood	None	3.00	6.66	-669	11	27	32	4.9	5	13	2	53	361	54
Flood	None	3.02	6.79	-660	16	35	37	4.92	7	15	2	106	532	62
Flood*	None	2.93	6.81	-664	14	19	22	5.62	7	7	2	91	123	37
Flood	None	2.92	6.72	-665	12	22	25	4.54	10	8	2	117	188	42
Flood*	Add	3.33	6.73	-671	15	33	33	5.06	10	12	2	148	380	55
Flood	Add	3.29	6.79	-672	15	30	38	4.74	8	15	2	125	457	63
Flood	Add	3.19	6.66	-646	12	29	28	4.48	8	10	2	103	295	48
Flood	Add	3.17	6.89	-671	18	34	37	5.1	7	7	2	120	227	62

*pots with only one plant. All other pots contained two plants per pot (a hill)

Appendix A (cont)

Table 4. Raw data for rice Cu variables

Water	OM	Soil % OM	Soil pH	Soil Eh	Plant Dry Wt.			DPTA Zn Extract	Zn concentration			Zn Uptake		
					root	stem	grain		root	stem	grain	Root	stem	grain
					grams			Micrograms per gram	micrograms gram			milligrams per pot		
Drain	None	3.00	6.87	74	13	32	26	15	152	162	40	1973	5229	1067
Drain	None	3.09	6.95	79	18	41	20	13	125	125	38	2291	5108	783
Drain	None	3.43	6.91	57	14	36	15	13	95	112	40	1364	3977	612
Drain	None	3.37	6.98	71	16	57	22	13	90	102	35	1418	3893	745
Drain	Add	3.42	6.88	60	18	41	20	15	130	123	42	2383	5040	851
Drain	Add	3.27	6.94	51	13	38	21	12	107	147	42	1461	5635	863
Drain	Add	2.98	6.97	68	11	29	20	13	98	152	40	1112	4330	800
Drain	Add	3.21	6.85	51	14	35	19	11	110	132	40	1525	4593	769
AWD	None	3.11	6.87	-543	14	27	30	6	177	30	25	2406	824	744
AWD	None	2.92	6.84	-544	15	36	35	5	132	23	20	1972	836	702
AWD	None	2.80	6.94	-546	17	37	34	7	133	23	22	2288	872	735
AWD	None	2.69	6.83	-515	14	32	31	5	135	23	30	1934	743	934
AWD	Add	3.38	6.8	-512	10	33	34	6	98	28	22	1012	922	741
AWD*	Add	3.33	6.68	-546	10	18	19	6	180	23	20	1720	425	383
AWD	Add	3.42	6.88	-533	10	30	37	6	130	25	20	1308	762	733
AWD	Add	2.70	6.88	-521	16	28	29	5	128	23	20	2116	658	588
Flood	None	3.00	6.66	-669	11	27	32	7	180	28	23	1918	768	756
Flood	None	3.02	6.79	-660	16	35	37	8	170	27	22	2708	946	803
Flood*	None	2.93	6.81	-664	14	19	22	8	140	20	25	1908	370	555
Flood	None	2.92	6.72	-665	12	22	25	6	158	23	23	1857	527	582
Flood*	Add	3.33	6.73	-671	15	33	33	6	258	25	22	3816	814	717
Flood	Add	3.29	6.79	-672	15	30	38	9	208	30	22	3125	914	818
Flood	Add	3.19	6.66	-646	12	29	28	9	198	25	23	2451	737	665
Flood	Add	3.17	6.89	-671	18	34	37	7	162	28	25	2906	963	924

*pots with only one plant. All other pots contained two plants per pot (a hill)

Appendix B.

Table 1. ANOVA table for response variables to treatments

	R2 adj	S	Normal AD (p) value	Equal Var (p) value	Adj Treat Seq SS	Adj Error Seq SS	Adj Treat MS	Adj Error MS	F Test	Treat p value
Soil pH	0.43	0.072	0.623	0.625	0.115	0.0926	0.0029	0.0051	4.45	0.008
[Fe Soil]	0.768	0.169	0.465	0.366	23316	5160	4663	287	16.3	0.000
[Mn Soil]	0.882	0.157	0.623*	0.029	43876	4456	8775	247	35.5	0.000
[Cu Soil]	0.972	0.0245	0.061	0.165	48.3	1.078	9.65	0.0598	161.3	0.000
[Zn Soil]	0.896	0.0107	0.612	0.764	232	20.5	46.4	1.1	40.7	0.000
[Fe Stem]	0.000	0.409	0.899*	0.750	8140	30184	1628	1677	0.97	0.462
[Mn Stem]	0.792	1.34	0.344	0.769	1661008	322115	332202	17895	18.6	0.000
[Cu Stem]	0.000	0.0297	0.100	0.353	15.9	159	3.17	8.83	0.36	0.870
[Zn Stem]	0.946	0.1223	0.545*	0.024	60579	2693	12116	150	81.0	0.000
Grain Wt	0.493	0.0504	0.725	0.099	695.5	457.5	139.1	25.4	5.47	0.003
Stem Wt	0.291	0.0515	0.636	0.635	383.4	477.5	76.7	26.5	2.89	0.003
Grain:Stem	0.8321	0.00107	0.641*	0.751	1.36	0.206	0.272	0.011	23.8	0.000

*Johnson S_U transformation for normal curve
Treatment df=5, Error df=18, total df=23

Appendix C

Table 1. Tukeys pairwise comparison (Mean Diff/*p value*)

	Soil pH	[Fe Soil]	[Mn Soil]	[Cu Soil]	[Zn Soil]	[FeStem]	[MnStem]	[CuStem]	[ZnStem]	GrainWt	StemWt	Grain/Stem
Treatment	SEDiff = 0.051	SEDiff = 11.9	SE Diff = 11	SE Diff = 0.173	SE Diff= 0.755	SE Diff= 29.0	SE Diff= 94.6	SE Diff= 2.10	SE Diff= 8.65	SE Diff= 3.56	SE Diff= 3.64	SE Diff= 0.0756
Drain NS vs Drain S	-0.0175/ 0.9992	-1.52/ 1.000	-0.980/ 1.000	-0.020/ 1.0000	-0.810/ 0.8859	-16.2/ 0.9924	56.2/ 0.9901	-0.4167/ 0.9999	13.3/ 0.6439	-0.830/ 0.999	-1.10/ 0.996	-0.0048/ 1.000
Drain NS vs AWDNS	-0.0575/ 0.8611	50.42/ 0.0059	67.6/ 0.0001	2.310/ 0.0000	-7.870/ 0.0000	-2.50/ 1.0000	705.4/ 0.000	1.250/ 0.9901	-100/ 0.0000	11.55/ 0.044	-3.65/ 0.9111	0.4096/ 0.0005
Drain NS vs AWD S	-0.1175/ 0.2380	69.04/ 0.0002	66.6/ 0.0002	2.74/ 0.0000	-7.655/ 0.0000	-30.8/ 0.8889	557.5/ 0.0002	1.250/ 0.9901	-100/ 0.0000	8.93/ 0.174	-9.42/ 0.1513	0.5098/ 0.000
Drain NS vs FloodfNS	-0.182/ 0.0214	73.80/ 0.0001	67.6/ 0.0001	3.37/ 0.0000	-6.185/ 0.0000	-41.25/ 0.7126	435.4/ 0.0026	1.667/ 0.9652	-100/ 0.0000	8.212/ 0.2427	-10.85/ 0.0736	0.5575/ 0.000
Drain NS vs Flood S	-0.160/ 0.0525	60.91/ 0.0009	121.1/ 0.000	3.22/ 0.0000	-4.385/ 0.0002	12.08/ 0.9981	540.0/ 0.0003	1.667/ 0.9652	-97.9/ 0.0000	13.14/ 0.018	-5.16/ 0.7172	0.4998/ 0.0001
Drain S vs AWD NS	-0.040/ 0.9660	51.94/ 0.0045	68.6/ 0.0001	2.330/ 0.0000	-7.060/ 0.0000	13.75/ 0.9965	649.2/ 0.0000	1.667/ 0.9652	-113.3/ 0.0000	12.38/ 0.0276	-2.551/ 0.9795	0.4144/ 0.0004
Drain S vs AWDS	-0.100/ 0.3948	70.56/ 0.0002	67.6/ 0.0001	2.760/ 0.0000	-6.845/ 0.0000	-14.58/ 0.9954	501.2/ 0.0006	1.667/ 0.9652	-113/ 0.0000	9.765/ 0.115	-8.323/ 0.250	0.5147/ 0.0000
Drain S vs Flood NS	-0.165/ 0.0431	75.32/ 0.0001	68.6/ 0.0001	3.390/ 0.0000	-5.375/ 0.0000	-25.00/ 0.9505	379.2/ 0.0091	2.083/ 0.9149	-114/ 0.0000	9.043/ 0.165	-9.752/ 0.1289	0.5623/ 0.0000
Drain S vs Flood S	-0.142/ 0.1015	62.42/ 0.0007	122.1/ 0.0000	3.240/ 0.0000	-3.575/ 0.0020	28.33/ 0.9189	483.7/ 0.0009	2.083/ 0.9149	-113/ 0.000	13.97/ 0.0109	-4.059/ 0.8691	0.5046/ 0.0000
AWDNS vs AWD S	-0.06/ 0.8393	18.62/ 0.6357	-1.01/ 1.0000	0.430/ 0.1800	0.215/ 0.9997	-28.33/ 0.9189	-147.9/ 0.6306	0.0000/ 1.000	-0.000/ 1.000	-2.620/ 0.975	-5.771/ 0.6181	0.1002/ 0.7677
AW NS vs Flood NS	-0.125/ 0.1869	23.38/ 0.4048	-0.025/ 1.0000	1.060/ 0.0001	1.685/ 0.2718	-38.75/ 0.7609	-270.0/ 0.0933	0.4167/ 1.0000	-0.4167/ 1.0000	-3.342/ 0.931	-7.20/ 0.3918	0.1479/ 0.4029
AWD NS vs Flood S	-0.1025/ 0.3693	10.48/ 0.9477	53.475/ 0.0017	0.910/ 0.0007	3.485/ 0.0025	14.58/ 0.9954	-165.4/ 0.5198	0.4167/ 0.1000	2.083/ 0.9999	1.590/ 0.997	-1.508/ 0.9982	0.0902/ 0.8346
AWD S vs Flood NS	-0.065/ 0.7911	4.755/ 0.9985	0.985/ 1.0000	0.630/ 0.0195	1.470/ 0.4079	-10.42/ 0.9991	-122.1/ 0.7862	0.4167/ 1.0000	-0.4167/ 1.0000	-0.722/ 0.999	-1.429/ 0.9986	0.4766/ 0.9871
AWD S vs Flood S	-0.0425/ 0.9562	-8.140/ 0.9820	54.48/ 0.0014	0.480/ 0.1080	3.270/ 0.0046	42.92/ 0.6791	-17.5/ 1.0000	0.4167/ 1.0000	2.083/ 0.9999	4.210/ 0.999	4.264/ 0.8448	-0.0101/ 1.0000
Flood NS vs Flood S	0.0225/ 0.9975	-12.89/ 0.8842	53.5/ 0.0017	-0.150/ 0.9496	1.800/ 0.2135	53.33/ 0.4658	104.6/ 0.8728	0.0000/ 1.0000	2.500/ 0.9997	4.932/ 0.736	5.693/ 0.6310	-0.058/ 0.9703

APPENDIX D

Table 1. The p values for grain weight vs. micronutrient content among water groups.

	P value	R ²	Normality (p value)	Equal variance (p value)
Fe Stem	0.014*	0.756	0.769	0.321
water	0.320			
Fe stem X water	0.013*			
Mn stem	0.809	0.671	0.570	0.087
water	0.293			
Mn stem X water	0.203			
Cu stem	0.029*	0.679	0.637	0.370
Cu water	0.629			
Cu stem X water	0.966			
Zn stem	0.015*	0.740	0.287	0.282
Zn water	0.212			
Zn stem X water	0.017*			

*indicates significance at the 0.05 alpha level